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Purification and Characterization of the Reconstitutively Active Adenine Nucleotide Carrier from Maize Mitochondria[1]

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The adenine nucleotide carrier from maize (*Zea mays* L. cv B 73) shoot mitochondria was solubilized with Triton X-100 and purified by sequential chromatography on hydroxyapatite and Matrex Gel Blue B in the presence of cardiolipin and asolectin. Sodium dodecyl sulfate-gel electrophoresis of the purified fraction showed a single polypeptide band with an apparent molecular mass of 32 kD. When reconstituted in liposomes, the adenine nucleotide carrier catalyzed a pyridoxal 5′-phosphate-sensitive ATP/ATP exchange. It was purified 168-fold with a recovery of 60% and a protein yield of 0.25% with respect to the mitochondrial extract. Among the various substrates and inhibitors tested, the reconstituted protein transported only ADP, ATP, GDP, and GTP, and was inhibited by atractyloside, bongkrekate, phenylisothiocianate, pyridoxal 5′-phosphate, and mersalyl (but not *N*-ethylmaleimide). Maximum initial velocity of the reconstituted ATP/ATP exchange was determined to be 2.2 μmol min^{-1} mg^{-1} protein at 25°C. The half-saturation constants and the corresponding inhibition constants were 17 μM for ATP, 26 μM for ADP, 59 μM for GTP, and 125 μM for GDP. The activation energy of the ATP/ATP exchange was 48 kilojoule/mol between 0 and 15°C, and 22 kilojoule/mol between 15 and 35°C. Partial amino acid sequences showed that the purified protein was the product of the ANT-G1 gene sequenced previously (B. Bathgate, A. Baker, C.J. Leaver [1989] Eur J Biochem 183: 303–310).

The inner membrane of plant mitochondria contains at least 10 specific carrier systems for the transport of metabolites (Day and Wiskich, 1984; Hanson, 1985; Heldt and Flügge, 1987; Oliver, 1987; Douce and Neuburger, 1989; Pozueta-Romero et al., 1991). The main properties of all of these carriers have been studied in intact mitochondria. However, essential for the identification of a transport protein and for its detailed functional and structural characterization is the purification and reconstitution of the purified protein in artificial membranes. So far, six of the mitochondrial metabolite carriers described in plants have been partially purified and reconstituted into liposomes: the dicarboxylate (Vivekananda et al., 1988), glutamate/aspartate (Vivekananda and Oliver, 1989), monocarboxylate (Vivekananda and Oliver, 1990), α-ketoglutarate (Genchi et al., 1991), tricarboxylate (McIntosh and Oliver, 1992), and phosphate (McIntosh and Oliver, 1994) carriers.

The ADP/ATP carrier, also called the adenine nucleotide translocator, is an intrinsic protein of the inner mitochondrial membrane that exchanges cytoplasmic ADP for ATP synthesized inside the mitochondrion, and is therefore an essential component of the apparatus presiding over the aerobic energy metabolism in the cell (Klingenberg, 1985; Vignais et al., 1985). Since the activity of the ADP/ATP translocator present in the inner envelope membrane of chloroplasts is very low (Heldt and Flügge, 1992), it is believed that in plant cells, even during photosynthetic metabolism, the cytosolic ATP pool is to a large extent maintained by oxidative phosphorylation (Krömer, 1995). The ADP/ATP carrier from pea leaves has previously been reconstituted into liposomes from a Triton X-100 total mitochondrial extract (Schünemann et al., 1993). Although purification of this transport system from plants has not yet been achieved, the primary structure of the plant ADP/ATP translocator is known from DNA sequencing. The open reading frames of the cDNAs for the ADP/ATP carriers in maize (Bathgate et al., 1989; Winning et al., 1991), potato (Emmerman et al., 1991), rice (Hashimoto et al., 1993), and wheat (Iacobazzi and Palmieri, 1995; Iacobazzi et al., 1996) encode proteins related in sequence to a number of mammalian mitochondrial carriers, including the ADP/ATP, phosphate, oxoglutarate, and tricarboxylate carriers, and the uncoupling protein from brown fat (Kuan and Saier, 1993; Palmieri, 1994). Unlike the ADP/ATP carriers in mammals and fungi, the plant adenine nucleotide translocator is synthesized with an N-terminal extension that is cleaved upon import into mitochondria (Mozo et al., 1995). Recently, cDNA clones encoding a mitochondrial malate translocator have been isolated from *Panicum miliaceum* L. (Taniguchi and Sugiyama, 1996). These clones

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Abbreviations: ANT-G, adenine nucleotide translocator gene; CNBr, cyanogen bromide.

show high similarity to the bovine mitochondrial 2-oxoglutarate/malate translocator (Runswick et al., 1990).

In this paper we describe the purification of the ADP/ATP carrier from maize (*Zea mays* L. cv B 73) shoot mitochondria using functional reconstitution as a monitor of the carrier activity during isolation. Upon SDS-gel electrophoresis the purified ADP/ATP transport protein appears to be a single polypeptide with an apparent molecular mass of 32 kD. Partial amino acid sequences show that the isolated protein from maize mitochondria is the product of the ANT-G1 gene characterized by Bathgate et al. (1989). The functional properties of the purified carrier incorporated into liposomes are also described here.

MATERIALS AND METHODS

Maize (*Zea mays* L. cv B 73) kernels, a generous gift from KWS Italia (Monselice, Italy), were surface-sterilized for 2 min in 1% (w/v) sodium hypochlorite and then rinsed in distilled water. Seeds were allowed to imbibe in water overnight and sown in damp granules of expanded clay. Seedlings were grown for 4 to 5 d in a dark-controlled environmental chamber at 30°C and 95% RH before harvesting. Hydroxyapatite (Bio-Gel HTP) was obtained from Bio-Rad; Triton X-100, acrylamide, and *N,N'*-methylenebisacrylamide from Serva (Paramus, NJ); Dowex AG1-X8 (100–200 mesh), egg-yolk phospholipids (lecithin from eggs), and Amberlite XAD-2 from Fluka; Matrex Gel Blue B from Amicon (Beverly, MA); [^{3}H]ATP from Amersham; cardiolipin from Avanti-Polar Lipids (Alabaster, AL); Sephadex G-75 from Pharmacia; and PVDF membranes from Applied Biosystems. All other reagents were of the highest purity commercially available.

Isolation and Purification of Maize Mitochondria

Maize shoots were disrupted with a mixer (Braun) in 3 volumes of ice-cold 0.4 M Suc, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.1% (w/v) BSA, 0.05% (w/v) Cys three times for approximately 1 min each. The homogenate was filtered through a layer of nylon and centrifuged for 20 min at 10,000*g*. The pellet was resuspended in a washing medium containing 0.3 M Suc, 5 mM Tris-HCl, pH 7.2, and centrifuged for 5 min at 1,000*g*. The decanted supernatant was layered onto a discontinuous Suc gradient, and purification of the mitochondria was carried out according to Douce et al. (1972), except that 5 mM Tris-HCl, pH 7.2, was used instead of 10 mM phosphate buffer in all purification steps. Purified mitochondria were suspended at a concentration of 15 to 18 mg protein per mL of washing medium, pH 7.2, frozen in liquid nitrogen and stored at −80°C.

Purification of the ADP/ATP Carrier

Maize shoot mitochondria were solubilized in buffer A (3% Triton X-100 [w/v], 20 mM Na_2SO_4, 1 mM EDTA, and 10 mM Pipes, pH 7.0) at a final concentration of 15 mg protein per mL of buffer. After 10 min at 0°C the mixture was centrifuged at 105,000*g* for 15 min; then, 450 μL of ultracentrifuge supernatant (extract) supplemented with cardiolipin (1 mg in 50 μL of buffer A) was applied to cold hydroxyapatite columns (Pasteur pipettes containing 600 mg of dry material) and eluted with the solubilization buffer. The first milliliter of the eluates from two hydroxyapatite columns were pooled and applied on cold Matrex Gel Blue B columns (Pasteur pipettes containing 1 mL of resin) pre-equilibrated with buffer B (0.1% Triton X-100, 10 mM Na_2SO_4, 1 mM EDTA, and 5 mM Pipes, pH 7.0). Elution was performed with 2 mL of buffer B, followed by 2 mL of buffer B supplemented with 2 mg/mL of asolectin, in which pure ADP/ATP carrier was collected in the first 1.5 mL. The Matrex Gel Blue B had been previously washed sequentially with 4 mL of 8 M urea/0.5 M NaOH, 4 mL of distilled water, and 4 mL of buffer B. The urea/base wash and the water wash were carried out at room temperature, whereas the buffer B wash was performed in a cold room. All of the other operations were performed at 4°C.

Reconstitution of the ADP/ATP Carrier in Liposomes

Protein eluates were reconstituted by removing the detergent with a hydrophobic column (Palmieri et al., 1995). In this procedure, the mixed micelles containing detergent, protein, and phospholipids were repeatedly passed through the same Amberlite XAD-2 column (Fluka). The composition of the initial mixture used for reconstitution was: (a) 200 μL of the eluates of the different columns or 20 μL of the extract plus 180 μL of buffer A; (b) 100 μL of 10% Triton X-114; (c) 100 μL of 10% egg-yolk phospholipids in the form of sonicated liposomes prepared as described by Bisaccia et al. (1985); (d) 20 mM ATP or other substrates; and (e) 10 mM Pipes, pH 7.0, in a final volume of 700 μL. After vortexing, this mixture was passed 15 times through the same column (0.5 × 3.6 cm) pre-equilibrated with a buffer containing 10 mM Pipes, pH 7.0, and 20 mM of the substrate present in the starting mixture. All of the operations were performed at 4°C, except the passage through the column, which was carried out at room temperature.

Transport Measurements

To remove the external substrate, 650 μL of the proteoliposomal suspension was passed through a column (Sephadex G-75, Sigma) (0.7 × 15 cm) pre-equilibrated with 50 mM NaCl and 10 mM Pipes, pH 7.0. The eluted proteoliposomes (600 μL) were distributed in reaction vessels (150 μL) and used for transport measurements by the inhibitor stop method (Palmieri and Klingenberg, 1979). Transport was begun by the addition of 10 μL of [^{3}H]ATP at the indicated concentrations. The exchange was stopped after the desired time interval by adding 10 μL of 350 mM pyridoxal 5'-phosphate. In control samples the inhibitor was added with the labeled substrate. The assay temperature was 25°C. The external radioactivity was removed by passing the samples (160 μL) through an anion-exchange column (Dowex AG1-X8, acetate form, 0.5 × 5 cm). The liposomes eluted with 1.0 mL of 50 mM sodium acetate were collected in 4 mL of scintillation mixture, vortexed, and counted. The experimental values were corrected by subtracting the respective control. The pyridoxal 5'-

phosphate-insensitive radioactivity in the control samples was always less than 8% of the pyridoxal 5'-phosphate-sensitive radioactivity taken up during the transport assay. K_m and V_{max} values were determined by a computer-fitting program based on linear regression analysis.

Other Methods

Polyacrylamide slab gel electrophoresis of acetone-precipitated samples was performed in the presence of 0.1% SDS according to Laemmli (1970). A minigel system was used: gel sizes were 8 cm × 10 cm × 1.5 mm (thickness). The stacking gel contained 5% acrylamide and the separation gel contained 17.5% acrylamide with a ratio of acrylamide/bisacrylamide of 30:0.2 to give a high resolution of polypeptides with a molecular mass close to 30 kD. CNBr cleavage of purified ADP/ATP carrier was performed under argon for 4 h with an excess of CNBr (30 mg/mL) on the protein dissolved in 70% formic acid. For peptide analysis the discontinuous Tricine system of Schägger and von Jagow (1987) was employed. We used 16.5% acrylamide with an acrylamide/bisacrylamide ratio of 15.5:1, with 13% glycerol included in the separation gel. For protein sequencing the peptides were separated by the same method (Schägger and von Jagow, 1987), transferred to PVDF membranes, detected by staining with Coomassie blue, excised, and subjected to Edman degradation in a pulse liquid protein sequencer (model 477A, Applied Biosystems) equipped with an on-line phenylthiohydantoin-amino acids analyzer. Staining was performed by the silver nitrate method (Morrissey, 1981). Protein was determined by the Lowry method modified for the presence of Triton X-100 (Dulley and Grieve, 1975). All samples used for protein determination were subjected to acetone precipitation and redissolved in 1% SDS.

RESULTS

Purification of the Adenine Nucleotide Carrier

Maize shoot mitochondria were solubilized in Triton X-100 in the presence of cardiolipin and subjected to chromatography on hydroxyapatite followed by a second chromatography on Matrex Gel Blue B (Table I). The passage of the mitochondrial extract through hydroxyapatite led to a substantial purification of the adenine nucleotide carrier. About 95% of the proteins present in the extract were bound to this resin. In the hydroxyapatite eluate 71% of the total activity was recovered and the specific activity was increased 14-fold. For further purification, the hydroxyapatite pass-through was subjected to chromatography on Matrex Gel Blue B (see "Materials and Methods"). By this purification step, eluting the column with buffer B in the presence of asolectin, the specific activity of the reconstituted ATP transport was increased 12.6- and 168-fold with respect to that of hydroxyapatite eluate and mitochondrial extract, respectively. Approximately 40% of the total transport activity was recovered, with a protein yield of 0.25%.

Figure 1 shows an SDS-PAGE of hydroxyapatite pass-through (lane 2) and Matrex Gel Blue B eluate (lanes 3, 4, and 6) obtained from maize mitochondria solubilized with Triton X-100. The fraction in lane 2 was substantially purified with respect to mitochondrial extract (lane 1), although it still contained various protein bands with apparent molecular masses between 30 and 36 kD. Figure 1 (lane 6) shows that a single protein band with an apparent molecular mass of 32 kD was eluted from Matrex Gel Blue B in the presence of asolectin. The fractions in lanes 3 and 4 of Matrex Gel Blue B were not active in reconstituted ATP transport.

Properties of the Reconstituted Adenine Nucleotide Carrier

In all of the experiments the reconstituted system consisted of purified protein eluted in the presence of asolectin from Matrex Gel Blue B (Fig. 1, lane 6) and incorporated in liposomes. Figure 2 illustrates the time course of the pyridoxal 5'-phosphate-sensitive [^{3}H]ATP uptake by proteoliposomes loaded with unlabeled ATP (20 mM). The uptake of ATP increased linearly with time for about 5 min at a rate of 1900 nmol min^{-1} mg^{-1} protein at 25°C (at 0.1 mM [^{3}H]ATP). In the absence of internal substrate, ATP uptake was totally absent. Likewise, there was no activity without incorporation of the carrier protein or with incorporation of heat-denatured carrier protein (2 min at 100°C) into the liposomes.

The time course of [^{3}H]ATP/ATP exchange, as shown in the inset to Figure 2, represents an exponential approach to isotopic equilibrium, which is demonstrated by the straight line obtained by plotting the natural logarithm of the fraction of equilibrium $ATP_{max}/(ATP_{max} - ATP_t)$ against time. This means that the exchange of ATP in proteoliposomes follows first-order kinetics. The first-order rate constant k, extrapolated from the slope of the logarithmic plot, was 0.06 min^{-1}.

The substrate specificity of [^{3}H]ATP with respect to intraliposomal counteranions was investigated in proteolipo-

Table I. *Purification of the adenine nucleotide carrier from maize mitochondria*

The proteoliposomes were loaded with 20 mM ATP and the exchange was started by the addition of 0.1 mM external [^{3}H]ATP.

Purification Step	Protein	Specific Activity	Total Activity	Purification
	mg	*nmol 10 min^{-1} mg^{-1} protein*	*nmol 10 min^{-1}*	*-fold*
Extract	6.00	60	360	1
Hydroxyapatite	0.32	800	256	14
Matrex Gel Blue B[a]	0.015	10,092	151	168

[a] Eluate with buffer B plus asolectin (1.5 mL).

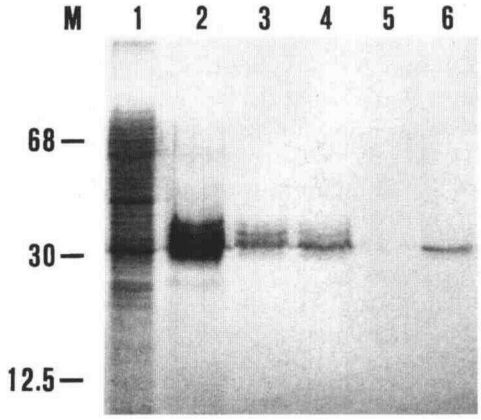

Figure 1. Purification of ADP/ATP carrier from maize mitochondria. Results of SDS gel electrophoresis of fractions obtained by hydroxyapatite and by Matrex Gel Blue B of maize mitochondria solubilized with Triton X-100 are shown. Lane M, Protein markers (from top to bottom: BSA, carbonic anhydrase, and Cyt *c*); lane 1, Triton X-100 mitochondrial extract (180 μg in 15 μL); lane 2, hydroxyapatite eluate (26 μg in 80 μL); lane 3, Matrex Gel Blue B pass-through (5.1 μg in 200 μL); lane 4, Matrex Gel Blue B eluate with buffer B (4.5 μg in 200 μL); lane 6, Matrex Gel Blue B eluate with buffer B plus asolectin (2 μg in 200 μL).

somes loaded with a variety of substrates. The intraliposomal concentration of the anions used was 20 mM and the exchange time was 10 min. The data reported in Table II show that 0.1 mM [^{3}H]ATP could be transported against ATP, ADP, and, to a lesser extent, d-ATP. Surprisingly, labeled ATP could also be exchanged for GTP and GDP, although the extent of the heterologous ATP/GTP and ATP/GDP exchanges after 10 min of incubation was only 60 and 31%, respectively, that of the homologous ATP/ATP exchange. In contrast, labeled ATP did not significantly exchange against AMP or cytosine-, thymidine-, and uracil-nucleotides, or against substrates of other mitochondrial carriers such as citrate, 2-oxoglutarate, malate, phosphate, and (not shown) pyruvate, glutamate, aspartate, oxaloacetate, Gly, and Ser. These results are in agreement with the narrow specificity of the ADP/ATP carrier as characterized in mitochondria (Earnshaw, 1977; Wiskich, 1977; Hanson, 1985; Klingenberg, 1985).

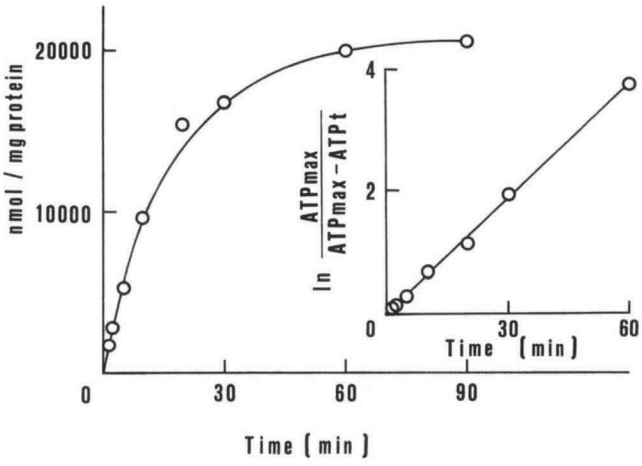

Figure 2. Time-course of ATP uptake in reconstituted liposomes. [^{3}H]ATP (0.1 mM) was added at time 0 to reconstituted liposomes containing 20 mM ATP. The inset represents the logarithmic plot of ln $ATP_{max}/(ATP_{max} - ATP_t)$, where ATP_{max} is the maximum ATP exchange mg^{-1} protein and ATP_t is the ATP exchange mg^{-1} protein at time *t*, according to the relation ln $ATP_{max}/(ATP_{max} - ATP_t) = k_t$.

Table II. *Dependence of ATP transport in reconstituted liposomes on internal substrates*

The proteoliposomes were loaded with the indicated substrates. Transport was initiated by adding 0.1 mM [^{3}H]ATP. The results are the mean of three experiments.

Internal Substrate (20 mM)	ATP Transport
	nmol 10 min^{-1} mg^{-1} protein
None (Cl^- present)	185
ATP	9940
ADP	9256
AMP	304
d-ATP	4816
GTP	5964
GDP	3100
CTP	206
CDP	175
TDP	184
UTP	172
UDP	177
Citrate	167
2-Oxoglutarate	173
Malate	178
Phosphate	190

The sensitivity of the reconstituted ATP/ATP exchange to externally added substrates and inhibitors was also investigated. Table III (experiment 1) shows that ATP/ATP exchange was inhibited strongly by ATP and ADP, and less efficiently by d-ATP, GTP, and GDP. In contrast, AMP, CTP, CDP, TDP, UTP, UDP, citrate, 2-oxoglutarate, malate, and phosphate had no effect. In addition, ATP/ATP exchange (see Table III, experiment 2) was inhibited by atractyloside, carboxyatractyloside, and bongkrekate, which are known inhibitors of the adenine nucleotide transporter in both plant and animal mitochondria (Vignais et al., 1976; Earnshaw, 1977; Wiskich, 1977; Hanson, 1985; Klingenberg, 1985). The data reported in Table III also show that the sulfhydryl reagents mersalyl and *p*-hydroxymercuribenzoate (but not *N*-ethylmaleimide), as well as the lysyl-specific reagents phenylisothiocyanate and pyridoxal 5′-phosphate, strongly inhibited the reconstituted ATP exchange. In contrast, inhibitors of other mitochondrial metabolite carriers such as 1,2,3-benzenetricarboxylate (Table III), phthalonate, buthylmalonate, and phenylsuccinate (not shown) had no significant effect.

Temperature Dependence of [^{3}H]ATP/ATP Exchange

Figure 3 shows the temperature dependence of the rate of ATP/ATP exchange. The Arrhenius plot shows two linear ranges with different slopes. The break point oc-

Table III. *Sensitivity of ATP/ATP exchange in reconstituted liposomes to externally added substrates and inhibitors*

The proteoliposomes were loaded with 20 mM ATP and the exchange was started by adding 0.1 mM [^{3}H]ATP. The −SH reagents were added 2 min before the labeled substrate at 2 mM concentration. The other inhibitors and the external anions were added with [^{3}H]ATP at a concentration of 10 mM, except atractyloside and carboxyatractyloside (100 μM), and bongkrekate (10 μM). The control values of uninhibited ATP exchange were 9120 and 8590 nmol 10 min^{-1} mg^{-1} protein in experiments 1 and 2, respectively. The data are the mean of three experiments.

Addition	Percent Inhibition
Experiment 1	
ATP	98
ADP	88
AMP	8
d-ATP	68
GTP	56
GDP	25
CTP	4
CDP	6
TDP	3
UTP	8
UDP	7
Citrate	5
2-Oxoglutarate	3
Malate	8
Phosphate	7
Experiment 2	
1,2,3-Benzenetricarboxylate	9
Atractyloside	94
Carboxyatractyloside	100
Bongkrekate	91
Mersalyl	100
N-Ethylmaleimide	4
p-Hydroxymercuribenzoate	92
Phenylisothiocyanate	93
Pyridoxal 5′-phosphate	100

curred at 15°C. The activation energies as derived from the slopes were 48.4 and 21.6 kJ/mol between 0 and 15°C, and 15 and 35°C, respectively. The break in the Arrhenius plot is somewhat lower than that obtained with the reconstituted ADP/ATP carrier from bovine heart mitochondria (Krämer, 1982). This may be due to the different composition of the liposomes.

K_m and V_{max} Values of ATP Transport

To obtain the basic kinetic data of the adenine nucleotide carrier from maize shoot mitochondria the dependence of the exchange rate on substrate concentration was studied by changing the concentration of externally added [^{3}H]ATP at a constant internal concentration of 20 mM ATP. In 12 experiments for the ATP substrate at 25°C an average of 17.8 ± 2.9 μM for the K_m and 2.15 ± 0.53 μmol min^{-1} mg^{-1} protein for the V_{max} was determined.

Inhibition by ADP and Guanine Nucleotides

The inhibition of the reconstituted ATP/ATP exchange by ADP, GTP, and GDP was analyzed in the presence of different substrate concentrations. ADP, GTP, and GDP were all identified as competitive inhibitors with respect to ATP. K_i, which is calculated from double reciprocal plots of the rate of ATP/ATP exchange versus substrate concentrations, was found to be 26 ± 5.1 μM for ADP, 59 ± 6.7 μM for GTP, and 125 ± 9.4 μM for GDP (four experiments for each nucleotide).

Partial Protein Sequencing

The purified protein and the peptides obtained by fragmenting the protein with CNBr were transferred to PVDF membranes and sequenced by Edman degradation. The N-terminal sequence of the intact protein was APAE-KGGK. Two abundant CNBr cleavage fragments showed the following N-terminal sequences: FAGNLASG and MT-SGEAVKYKSSLDA. These three sequences were present in the deduced amino acid sequence of the product of the maize adenine nucleotide translocator gene sequenced by Bathgate et al. (1989). The determination of the N-terminal sequence of the purified protein shows that the ADP/ATP carrier from maize is synthesized with an N-terminal extension of 19 amino acids, which is absent in the mature protein.

DISCUSSION

The isolation of mitochondrial metabolite carriers from plants appears to be more difficult than with mammalian

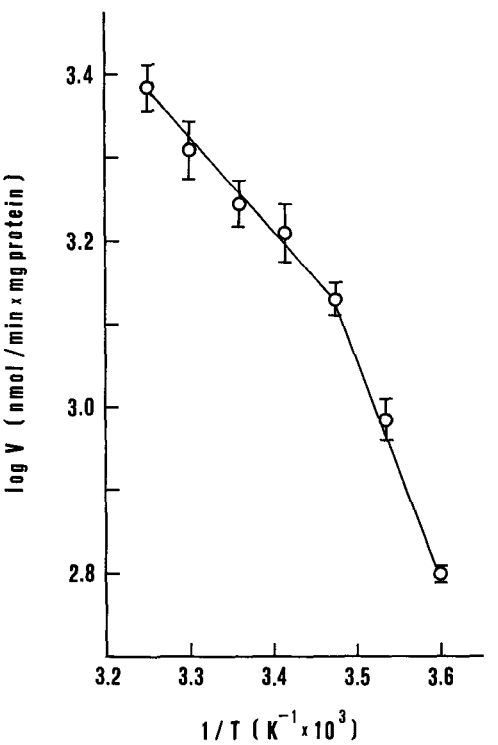

Figure 3. Arrhenius plot of the temperature dependence of the reconstituted ATP/ATP exchange activity. [^{3}H]ATP (0.1 mM) was added to proteoliposomes containing 20 mM ATP, which were incubated for 1 min at the indicated temperatures. The exchange activity, *V*, is expressed in nmol min^{-1} mg^{-1} protein. The means ± SD of four experiments are reported.

carriers. So far, none of these transporters has been purified to homogeneity. The procedure developed for the isolation of the ADP/ATP carrier from bovine heart (Krämer and Klingenberg, 1979) does not result in a pure preparation when used with plant mitochondria (G. Genchi, F. Bisaccia, and F. Palmieri, unpublished data).

The results reported in this paper represent, to our knowledge, the first report of a procedure that yields a highly purified preparation of functional adenine nucleotide transport protein from plant mitochondria. The highly purified nature of the final ADP/ATP carrier fraction is demonstrated by the SDS polyacrylamide gel electrophoretic analysis, which indicates the presence of a single mitochondrial band with an apparent molecular mass of 32 kD. This value falls into the very narrow range of apparent molecular masses between 28 and 34 kD shown by all mitochondrial metabolite carriers isolated so far (Palmieri, 1994). From the data in Table I it can be calculated that the ADP/ATP carrier represents only about 0.6% of the total protein present in maize mitochondria. This value is substantially less than that estimated for this translocator in bovine heart mitochondria (Klingenberg, 1985). Plant mitochondria have a very high Gly decarboxylase activity, and this enzyme makes up an important share of the mitochondrial protein (Douce and Neuburger, 1989). This feature and/or metabolic differences between plant and animal tissues may be responsible for the lower relative portion of the ADP/ATP carrier in maize. It should be stressed, however, that in the present paper the aim was to achieve optimal purification, not optimal yield. Therefore, the exact content of the adenine nucleotide translocator in the inner mitochondrial membrane of maize shoots relative to Cyt *a*, for example, remains to be determined.

The conclusion that the 32-kD polypeptide that we purified from maize mitochondria is in fact the adenine nucleotide carrier is supported by the following evidence. First, the three partial amino acid sequences that we determined in the purified protein correspond exactly to the amino acid sequence of the ADP/ATP carrier deduced from the ANT-G1 gene sequenced by Bathgate et al. (1989) in maize. Second, upon incorporation into liposomes, the purified material catalyzed a very active [^{3}H]ATP/ATP or [^{3}H]ATP/ADP exchange. Third, upon reconstitution, the purified transporter exhibited a substrate specificity (Table II) and an inhibitor sensitivity (Table III) that are very similar to those observed for the adenine nucleotide transport system in animal and plant mitochondria (Vignais et al., 1976; Earnshaw, 1977; Wiskich, 1977; Hanson, 1985; Klingenberg, 1985). We found only one major difference between plant and animal mitochondria: the nucleotide carrier from maize mitochondria transports not only ADP and ATP (like the ADP/ATP carrier from animal mitochondria), but also GDP and GTP (although less efficiently). Brustovetski and Klingenberg (1994) recently demonstrated that GDP and GTP bind to the ADP/ATP carrier from beef heart mitochondria (although they are not transported), and that this binding is increased by mersalyl. This finding suggests that the maize transporter does not differ from the animal homolog in substrate binding, but rather in the translocation channel. The translocation path or channel in the adenine nucleotide carrier in plants is clearly different from the one in the homologous carrier in animals, since in addition to ADP and ATP, guanine nucleotides are also translocated through the maize carrier protein. We hope that the purification and functional reconstitution of the maize ADP/ATP carrier described here will provide a useful basis for further characterization and understanding of the molecular mechanism of nucleotide transport across the mitochondrial inner membrane.

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